

**REMARKS**

Claims 1-10 have been amended to improve clarity and to place the claims in typical U.S. format. Claims 11-17 have been added to further define the present invention.

Favorable consideration of the application as amended is respectfully requested.

The Examiner is invited to contact the undersigned by telephone if it is felt that a telephone interview would advance the prosecution of the present application.

Respectfully submitted,

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FOLEY & LARDNER  
Washington Harbour  
3000 K Street, N.W., Suite 500  
Washington, D.C. 20007-5143  
Telephone: (202) 672-5300  
Facsimile: (202) 672-5399

By  34371  
*Glenn Law*  
for Andrew F. Knight  
Registration No. 50,443

APPENDIX A

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Claims:

Claims 1-10 have been amended as follows:

1. (Amended) A specimen holder [(1)] for water-containing specimens for high-pressure freezing, the specimen holder [(1)] comprising at least two shaped parts [(2, 3; 2, 4)] detachably joinable to one another, [and] wherein the joined shaped parts [(2, 3; 2, 4) forming] form a receptacle [(6)] for [the] holding a specimen, wherein at least one of the shaped parts [(2; 3)] comprises a diamond.
2. (Amended) The specimen holder [(1)] as defined in Claim 1, wherein at least one of the shaped parts [(2; 3)] is [of] disk-shaped [configuration].
3. (Twice Amended) The specimen holder [(1)] as defined in Claim 1, wherein a spacer ring [(9)] fabricated from metal is provided between the shaped parts [(2; 3)].
4. (Amended) The specimen holder [(1)] as defined in Claim 3, wherein both shaped parts [(2; 3)] are [configured as] disk-shaped diamonds.
5. (Twice Amended) The specimen holder [(1)] as defined in Claim 1, wherein one of the shaped parts comprises a diamond and the other of the shaped [part (3)] parts is fabricated from metal, is [of] disk-shaped [configuration], and comprises a shaped-on bead [(8)] running around [the] a rim of the disk.
6. (Amended) The specimen holder [(1)] as defined in Claim 1, wherein the diamond comprises an orifice [(10)] for the delivery of high pressure.

7. (Twice Amended) The specimen holder [(1)] as defined in Claim 3, wherein the metal comprises one of gold, [or] aluminum, and [or] copper [is used the metal].

8. (Amended) The specimen holder [(1)] as defined in Claim [1] 3, wherein [both] each shaped [parts (2; 3) are of] part has a planar [configuration on their inwardly directed surfaces, and a] surface on a surface facing the receptacle, and the spacer [(9) running around the surfaces is arranged between those surfaces as a seal and in order to constitute] is configured to serve as a seal between said planar surfaces and to define the specimen receptacle [(6)].

9. (Twice Amended) The specimen holder [(1)] as defined in Claim 1, wherein the diamond is [configured as] polycrystalline CVD diamond.

10. (Twice Amended) The specimen holder [(1)] as defined in Claim 1, wherein at least one of the shaped parts [(2, 3, 4) is configured with] has an irregularly shaped external surface.

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~~SPECIMEN HOLDER FOR A HIGH PRESSURE FREEZING DEVICE~~

**U.S. PATENT APPLICATION**

**for**

**SPECIMEN HOLDER FOR A HIGH-PRESSURE FREEZING DEVICE**

**Inventor: Paul WURZINGER**

**SPECIMEN HOLDER FOR A HIGH-PRESSURE FREEZING DEVICE**

**CROSS REFERENCE TO RELATED APPLICATIONS**

~~\_\_\_\_\_~~ This[0001] This invention claims priority of the German patent application 100 65 143.7 which is incorporated by reference herein.

**FIELD OF THE INVENTION**

~~\_\_\_\_\_~~ The[0002] The invention concerns a specimen holder for a high-pressure freezing device, ~~according to the preamble of Claim 1.~~

**BACKGROUND OF THE INVENTION**

~~\_\_\_\_\_~~ For[0003] For purposes of the invention, a "high-pressure freezing device" is understood to be a freezing system for rapid freezing (vitrification) of water-containing specimens under high pressure. Devices of this kind are described in DE Patent 18 06 741, EP 0 853 238 A1, and EP 0 637 741 A1.

[0004] A high-pressure freezing device of this kind is being successfully marketed by the Applicant under the name "Leica EM HPF," and is depicted in the document "LEICA EM HPF, High Pressure Freezer, 1.K.-LEICA EM HPF-E-6/94, Juni 1994."

[0005] The "Leica EM HPF" high-pressure freezing device makes it possible to vitrify conventional specimens under a pressure of approximately 2000 bar at a cooling rate of  $10^3$ – $10^5$  K/s. With this known unit, the critical cooling phase from room temperature to -100°C lasts approximately 10 ms at the surface of the specimen (cooling rate of  $10^4$  K/s).

[0006]\_\_\_The cooling rate in the interior of the specimen depends exclusively on the physical properties of the specimen. In order to vitrify thicker biological or water-containing specimens, the specimen is exposed to high pressure.

[0007]\_\_\_Because of the elevation in pressure, a slower cooling rate is sufficient for vitrification. The cooling rate for vitrification of biological specimens is approximate  $10^5$ – $10^6$  K/s at standard pressure, but at 2000 bar the cooling rate is only  $10^3$ – $10^4$  K/s; in other words, under high pressure biological specimens can still be vitrified at cooling rates that are about a hundred times lower.

[0008]\_\_\_Metal holders that comprise at least two shaped parts detachably joinable to one another are used for freezing the specimens, the shaped parts joined to one another forming a chamber for reception of the specimens. Specimen holders of this kind are depicted in the aforementioned document "LEICA EM HPF, High Pressure Freezer, 1.K.-LEICA EM HPF-E-6/94, Juni 1994" and in the document "Balzers, Hochdruck-Gefriermaschine [High-pressure freezing machine] HPM 010, Balzers Union Aktiengesellschaft, no publication date."

[0009]\_\_\_In order to freeze the specimen, the specimen holder is clamped in the high-pressure freezing device in such a way that on the one hand specimen loss is prevented, and on the other hand a sufficient portion of the surface remains freely accessible for a cryogen, e.g., liquid nitrogen, to be sprayed onto it. An arrangement of this kind is depicted and described in unpublished DE 100 15 773.

[0010]\_\_\_From the known art of refrigeration and from the physical processes, it is also known that the cooling rate at the center of a biological specimen is determined only to a limited extent by the cooling rate at the surface. The essential feature in this context is that with rapid cooling rates at the surface, a kind of "saturation effect"

occurs, whereas lower cooling rates at the surface inevitably lead to poor freezing results. It is therefore essential that the cooling rate achievable by a unit be transferred in as undiminished a fashion as possible by the specimen holding system onto the surface of the biological specimen.

### **SUMMARY OF THE INVENTION**

~~It~~[0011] It is therefore the ~~an~~ object of the present invention to create a specimen holder for a high-pressure freezing device that transfers the achievable cooling rate in as undiminished a fashion as possible onto the surface of the specimen.

~~According to the present invention, this object is achieved by the features recited in the characterizing portion of Claim 1. Advantageous developments of the invention are the subject matter of the dependent claims.~~

[0012] The present invention provides for a specimen holder for water-containing specimens for high-pressure freezing, the specimen holder comprising at least two shaped parts detachably joinable to one another, wherein the joined shaped parts form a receptacle for holding a ~~The invention is characterized in that one of the shaped parts is made of diamond or at least~~specimen, wherein at least one of the shaped parts comprises a diamond. Diamond possesses not only extreme hardness but also the advantage of low specific heat and very good thermal conductivity.

[0013] In a further embodiment of the invention, at least one of the shaped parts, preferably the shaped part fabricated of diamond, is of disk-shaped configuration. A spacer ring fabricated from metal, which determines the depression necessary for specimen reception, is

provided between the two shaped parts. ~~Said~~The spacer ring can also assume the sealing function in this context.

[0014]\_\_\_Provision can, of course, also be made for only one of the shaped parts to be fabricated of diamond or for the shaped part to comprise a mounted diamond. In this case the depression for specimen reception can be accomplished by the mount of the diamond or by a corresponding configuration of the other shaped part. A preferred exemplary embodiment is a specimen holder having one disk-shaped shaped part made of diamond, and one disk-shaped one made of metal. The metal shaped part has a circumferential shaped-on bead at the rim.

[0015]\_\_\_In a further embodiment of the specimen holder, the diamond or one of the shaped parts can be equipped with an orifice for the delivery of high pressure.

[0016]\_\_\_It has proven to be advantageous in the context of such specimen holders to use gold or aluminum or copper as the metal, and to utilize a commercially available polycrystalline CVD industrial diamond as the diamond.

[0017]\_\_\_In a further embodiment of the invention, both shaped parts are of planar configuration on their inwardly directed surfaces. A spacer running around the surfaces is arranged between those surfaces as a seal and in order to constitute the specimen receptacle. Differently dimensioned receptacles for the specimens can easily be produced by way of differently dimensioned spacers.

### **BRIEF DESCRIPTION OF THE DRAWINGS**

\_\_\_\_\_The[0018]\_\_\_The invention is depicted and explained in more detail, in several exemplary embodiments, on the basis of the schematic drawings in which:



~~FIG. 1 shows~~[0019] Fig. 1 shows a sectioned depiction of the specimen holder having two shaped parts manufactured from diamond and a circumferential metal ring;

~~FIG. 2 shows~~[0020] Fig. 2 shows a sectioned depiction of the specimen holder having one shaped part made of diamond and one shaped part made of metal;

~~FIG. 3 shows~~[0021] Fig. 3 shows a sectioned depiction of the specimen holder having two shaped parts made of diamond and an additional shaped part with a high pressure delivery line;

~~FIG. 4 shows~~[0022] Fig. 4 shows a sectioned depiction of the specimen holder having one shaped part made of diamond and another shaped part with a high pressure delivery line; and

~~FIG. 5 shows~~[0023] Fig. 5 shows a sectioned depiction of the specimen holder having one shaped part made of diamond and a shaped part forming a sample receptacle and a seal.

### **DETAILED DESCRIPTION OF THE INVENTION**

~~FIG.~~[0024] Fig. 1 shows a specimen holder 1 having a shaped part 2 and a shaped part 3 arranged opposite ~~the latter.~~each other. Both shaped parts 2 and 3 are produced from diamond and are configured as disk-shaped plates. A spacer ring 9, which preferably is made of metal, is provided between the two shaped parts 2 and 3. The metal used here is preferably gold, copper, or aluminum. The size of spacer ring 9 determines the size of sample receptacle 6. It is thus easy to achieve sample receptacles 6 of different sizes by way of differently dimensioned spacer rings 9.

[0025] Specimen receptacle 6 is isolated via spacer ring 9 when clamped into a clamping device (not depicted) of a high-pressure freezing device.

~~FIG.~~[0026] ~~Fig.~~ 2 shows a further exemplary embodiment of specimen holder 1 having one shaped part 2 fabricated of diamond and an oppositely arranged shaped part 3 produced from metal. Shaped part 3 has, at ~~the~~its rim, shaped-on beads 8 extending around the rim that assume the function of spacer ring 9 of ~~FIG.~~Fig. 1. Specimen receptacles 6 of different sizes can be implemented by different dimensioning of beads 8. A fixed specimen receptacle 6 is constituted by shaped-on beads 8, thereby facilitating insertion and removal of the specimen.

~~FIG.~~[0027] ~~Fig.~~ 3 shows a specimen holder 1 corresponding to the embodiment of ~~FIG.~~Fig. 1, where an orifice 10 for a downstream high-pressure device (not depicted here) ~~being~~is provided~~in this case in~~ shaped part 3. A further shaped part 4 having a high-pressure conduit 5 is associated with shaped part 3. High-pressure conduit 5 corresponds to orifice 10, which terminates in specimen receptacle 6. The necessary pressure can thereby be generated directly in specimen receptacle 6.

~~FIG.~~[0028] ~~Fig.~~ 4 shows an exemplary embodiment of specimen holder 1 corresponding to ~~FIG.~~ 1, ~~Fig.~~ 3, where shaped part 4 ~~here being~~is directly joined to shaped part 2 via seal 9. In this exemplary embodiment, shaped part 3 has been completely ~~replaced~~removed.

~~FIG.~~[0029] ~~Fig.~~ 5 shows an exemplary embodiment of specimen holder 1 according to ~~FIG.~~Fig. 4, where the encircling spacer ring 9 for sealing ~~having~~has been replaced~~here~~ by metal shaped part 3 having a bottom ~~8~~7 and the circumferentially shaped-on beads 8. This shaped part ~~3~~ corresponds to shaped part 3 of ~~FIG.~~ 2, ~~bottom 7 being in this case of the thinnest possible configuration.~~Fig. 2, and bottom 7 is very thin. Since shaped part 3 is, ~~in this case as well,~~ arranged removably in specimen holder 1, shaped part 3 can be removed separately. It is

thus easier for the specimens to be introduced into and removed from specimen holder 1 or specimen receptacle 6.

[0030] The shaped parts described above and fabricated from diamond, preferably from a polycrystalline CVD diamond, ~~can of course also~~ may comprise one or more mounted diamonds.

~~It is of course also within the context of the invention if the external surface~~

[0031] The external surfaces of the shaped elements ~~is of irregular configuration, so as thereby to constitute a greater~~ may be irregularly shaped, thereby constituting a greater surface area for spraying on liquid nitrogen.

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## **PARTS LIST**

- 1 Specimen holder
- 2 Shaped part
- 3 Shaped part
- 4 Shaped part
- 5 High-pressure conduit
- 6 Specimen receptacle
- 7 Bottom
- 8 Bead
- 9 Spacer ring
- 10 Orifice

**~~WHAT IS CLAIMED IS:~~**

1. ~~A specimen holder (1) for water-containing specimens for high pressure freezing, the specimen holder (1) comprising at least two shaped parts (2, 3; 2, 4) detachably joinable to one another, and the joined shaped parts (2, 3; 2, 4) forming a receptacle (6) for the specimen, wherein at least one of the shaped parts (2; 3) comprises a diamond.~~
2. ~~The specimen holder (1) as defined in Claim 1, wherein at least one of the shaped parts (2; 3) is of disk-shaped configuration.~~
3. ~~The specimen holder (1) as defined in Claim 1 or 2, wherein a spacer ring (9) fabricated from metal is provided between the shaped parts (2; 3).~~
4. ~~The specimen holder (1) as defined in Claim 3, wherein both shaped parts (2; 3) are configured as disk-shaped diamonds.~~
5. ~~The specimen holder (1) as defined in Claim 1 or 2, wherein the other shaped part (3) [is] fabricated from metal, [is] of disk-shaped configuration, and comprises a shaped-on bead (8) running around the rim.~~
6. ~~The specimen holder (1) as defined in Claim 1, wherein the diamond comprises an orifice (10) for the delivery of high pressure.~~
7. ~~The specimen holder (1) as defined in Claim 3 or 5, wherein gold or aluminum or copper is used the metal.~~
8. ~~The specimen holder (1) as defined in Claim 1, wherein both shaped parts (2; 3) are of planar configuration on their inwardly directed surfaces, and a spacer (9) running around the surfaces is arranged between those surfaces as a seal and in order to constitute the specimen receptacle (6).~~

9. ~~The specimen holder (1) as defined in at least one of the foregoing claims,  
wherein the diamond is configured as polycrystalline CVD diamond.~~
10. ~~The specimen holder (1) as defined in at least one of the foregoing claims,  
wherein at least one of the shaped parts (2, 3, 4) is configured with an  
irregularly shaped surface.~~

## ABSTRACT OF THE DISCLOSURE

\_\_\_\_\_A specimen holder~~(1)~~ for water-containing specimens for high-pressure freezing ~~is described, the specimen holder (1)~~ ~~comprising~~includes at least two shaped parts~~(2, 3)~~ detachably joinable to one another, ~~and~~where the joined shaped parts ~~(2, 3)~~ ~~forming~~form a receptacle~~(6)~~ for the specimen. At least one of the shaped parts~~(2, 3)~~ comprises a diamond.

~~(FIG. 1)~~